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44. Abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
45. The α-carbon coordinates for recombinant human IFN-γ D′ will be deposited with the Brookhaven Protein Data Bank. We thank S. K. Narula and staff for the expression of recombinant human IFN-γ D′ in *Escherichia coli,* G. F. Seelig and P. Reichert for preparation of purified protein, S. Senadhi for preparation of recombinant human IFN-γ D′ crystals, and C. Smith and B. Cole for assistance with data processing. Supported by NIH grant CA-13148 and NASA grant NAGW813.

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Fatal Sibling Aggression, Precocial Development, and Androgens in Neonatal Spotted Hyenas

LAURENCE G. FRANK, STEPHEN E. GLICKMAN, PAUL LICHT

Fatal neonatal sibling aggression is common in predatory birds but has not been previously reported in wild mammals. Spotted hyena females are strongly masculinized, both anatomically and behaviorally, apparently by high levels of androgens during ontogeny. Neonates display elevated androgen levels, precocial motor development, and fully erupted front teeth. Litters are usually twins, and siblings fight violently at birth, apparently leading to the death of one sibling in same-sex litters, whereas in mixed-sex litters both siblings usually survive.

SPOTTED HYENAS (*CROCUTA CROCUTA*) are the most abundant large terrestrial predators in sub-Saharan Africa. Their highly successful adaptations as social hunters include the behavioral dominance of females over males (*1*). Besides being heavier and more aggressive than males, females also have highly masculinized genitalia (*2*): the clitoris is greatly hypertrophied (the size of the male penis) and fully erectile. The vaginal labia are fused to form a scrotum. The urogenital canal traverses the clitoris, through which the female mates and gives birth.

A number of investigators have suggested (*3–5*) that "masculinization" of the female genitalia originated as a by-product of selection for secretion of androgens by female hyenas, resulting in increased body size and aggressiveness. The latter changes would be expected to enhance females' access to food during highly competitive feeding and thereby improve survival of offspring (*4, 5*). Selection would also operate on the by-products of such androgenization (for example, masculinization of genital morphology). Ability to participate in "meeting ceremonies," in which social cohesion is facilitated by mutual inspection of the erect genitals, was suggested as one such selective agent (*3*).

We describe another phenomenon that may have originated as a by-product of selection for female androgenization: extreme neonatal aggression that may culminate in the death of one member of the set of twins that normally constitute a litter.

Spotted hyenas weigh between 1.0 and 1.65 kg at birth and, unlike other carnivores (*6*), have fully erupted incisors and canines (Fig. 1). Their eyes are open and they are capable of strong, coordinated and highly directed behavior at birth. The stereotypic bite-shake attack that constitutes elevated aggression in adults commences in the first hour after birth. The prolonged gestation period of spotted hyenas, about 110 days, is presumably related to precocity. By contrast, in the striped hyena (*Hyaena hyaena*), gestation lasts about 90 days, incisors and canines erupt at 21 and 33 days, respectively, and the first social interactions, which are playful, appear at 30 days of age (*7*); females of this species do not exhibit masculinized genitalia.

Fig. 1. Dentition of spotted hyena on day of birth. Canines are 6 to 7 mm long; incisors 2 to 4 mm long.

We present data on fighting in five litters born at the University of California, Berkeley. The litters were videotaped for 12 to 24 hours per day from birth through 4 weeks of age: 121 to 147 hours per litter were analyzed (*8*). Unlike in the natural denning situation, the mother was accessible to the neonates 24 hours a day in the laboratory. Blood samples were taken at intervals through the first month after birth and analyzed (*9*) for androgens (testosterone and androstenedione).

Infants were delivered about 1 hour apart. The first born usually attacked the second within minutes of birth; in one case, the third born was attacked while still fully enclosed in its amniotic membrane. Aggression was most intense on the day of birth, falling rapidly thereafter (Fig. 2). Initially, all aggression consisted of bite-shakes, directed primarily at the neck and anterior dorsum. Fighting was initiated by the first born but quickly became mutual. One sibling established dominance over the other in the first days and thereafter bite-shakes were largely replaced by brief bites and threats. Beginning shortly after birth, a characteristic pattern of wounds could be observed on the back of the subordinate infant, although, in captivity, these only required medical treatment among triplets.

Plasma androgens are markedly elevated at birth in spotted hyenas. Plasma androstenedione (A) is similarly elevated in both sexes at birth, but it remains elevated during the first month in females whereas it falls progressively in males during the same time span (Fig. 3). Androstenedione, primarily of ovarian origin, remains the primary circulating androgen in nonpregnant adult females (*10*). In contrast, testosterone (T) is typically higher in males than in females throughout the first month after birth (Fig. 3).

The elevated androgens at birth are likely an extension of high levels in the maternal and fetal circulation during much of gesta-

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L. G. Frank and S. E. Glickman, Department of Psychology, University of California, Berkeley, CA 94720.
P. Licht, Department of Integrative Biology, University of California, Berkeley, CA 94720.

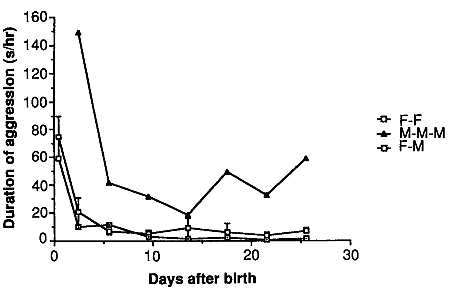

Fig. 2. Time course of aggression (while cubs were visible) in five litters of spotted hyena neonates during the first 27 days of life. Three litters are female-female pairs (mean ± SEM), one is a mixed-sex pair, and one is a litter of male triplets. Data were lumped in 4-day blocks, the first of which was divided into days 0 to 1 and days 2 to 3. Day 0 is the first 24 hours after birth. Data from days 0 to 1 are not available for the triplets. Repeated measures ANOVA based on data from the four twin litters indicated a statistically significant decline in duration of aggression [$F(7, 21) = 31.07$; $P < 0.0001$], frequency of aggression [$F(7, 21) = 4.36$; $P < 0.005$], and length of aggressive bouts [$F(7, 21) = 4.45$; $P < 0.005$].

tion. Lindeque and Skinner (*11*) reported 1.4 to 8 ng of T per milliliter of T in cardiac samples from several hyena fetuses (both sexes) between 31 and 80 days of gestation (estimated). We measured elevated T (~2 to 5 ng/ml) in several male and female fetuses (and in the maternal circulation) at 85 to 95 days gestation. Fetal levels of T are generally higher than at birth, especially in females. The presence of substantial plasma androgen levels during fetal and neonatal life would be compatible with the organization or activation of neonatal aggression by these steroids.

Wild spotted hyena females give birth at the mouth of a natal den, usually an abandoned aardvark burrow. Tunnels diverge from this den entrance, becoming smaller in diameter at greater distances from the opening, forcing even small hyenas to crawl on their carpals (*1*). This arrangement protects young from predation, but also prevents the mother from entering the burrow system; infants must emerge to nurse (*1*).

Cubs are brought to a communal den at 2 to 6 weeks of age. They are individually identifiable at this time, and recognizing sibling pairs is straightforward. Free ranging cubs can be sexed according to methods in (*12*). First litters are usually singletons (for example, five of six litters in our colony) and were not included in the following analysis. Subsequent litters are generally twins or triplets (12 of 13 in our colony: 5 male-female twins; 5 female-female twins, 1 all-male triplet; and 1 mixed-sex triplet). Twinning is also considered to be the norm from observations in other captive colonies and in nature (*1*).

The Talek clan, varying between 70 to 90 individuals, has been studied in the Masai Mara National Reserve in Kenya since 1979 (*5*). Contrary to expectations, of the 99 wild litters recorded at the time they were brought to the communal den, only 57 were twins and 42 were singletons. Among these 156 infants, the sex ratio did not differ from unity. However, among twins, the expected distribution of litter sex ratios was not observed. Given an equal probability of males and females at birth, the expected ratio of 1:2:1 for male-male:male-female:female-female two-cub litters should result in equal proportions of mixed- and same-sex litters (for 12 twins and triplets in our captive colony, the ratio of single- to mixed-sexed sets is 1:1 as expected). However, of the 33 sets of wild twins in which both members could be sexed before one died or disappeared, only 5 (15%) were same sex (4 sets of males, 1 set of females). This 5:28 ratio differs significantly from binomial expectation ($G = 17.68$, df = 1, $P < 0.001$). In the Kalahari, an entirely different ecosystem, eight out of nine twin litters were mixed sex (*13*).

Fighting observed in captive litters and the common pattern of wounds on the back observed in captive and wild litters were consistent with the hypothesis that most singleton litters at the communal den were originally same-sex twins. Because neonates do not emerge beyond the mouth of the natal den, it is difficult to observe infant behavior in the wild during this stage of development. But several direct observations in nature support the siblicide hypothesis: in a litter of twin females, collected at the natal den at approximately 1 week of age, one hyena weighed 2.0 kg, with sleek, unmarked fur, whereas its sibling weighed only 1.0 kg, presented extensive, infected wounds in the usual target areas on the back, and was badly dehydrated. The latter was nearly comatose and survived only through intensive feeding and wound treatment. A similar situation was observed in a set of twin males (estimated age 3 days), although the wounding was not as extensive and death was presumably days rather than hours away. Between 1979 and 1984, L. G. F. observed at least four cases of early litter reduction, from twins to singletons, in the field.

Even the relatively low frequency of twins may underestimate the extent of siblicide. Although no triplet litters have been observed at communal dens in Masai Mara, the occasional survival of same-sex siblings in the wild may be the result of triplet litters. Of 46 captive-bred litters produced in our colony and two zoological parks (*14*), 10 (22%) were triplets. Our experience suggests that if one cub bears the brunt of aggression by two others, the mid-ranking cub may be largely spared, resulting in its survival, regardless of sex.

In nature, the burrow denning habit appears to permit the dominant sibling to prevent the subordinate from gaining access to the mouth of the burrow, and hence the female to nurse, resulting in eventual death from starvation when both are the same sex. In most wild litters, priority of access to nursing allows the dominant sibling to become visibly larger than the subordinate (*15*), and the size differential appears to persist into adulthood. In captivity, where the infants had constant access to the mother, little size differential emerged.

Siblicidal brood reduction is common in predatory birds, in which it appears to tailor brood size to available resources (*16*). In spotted hyenas of the Masai Mara region, mixed-sex litters survive intact, so that food shortage clearly does not limit potential litter size to one. This area is rich in prey; hyena infants past the age of siblicide have not been observed to starve as long as their mother is alive. However, in other parts of

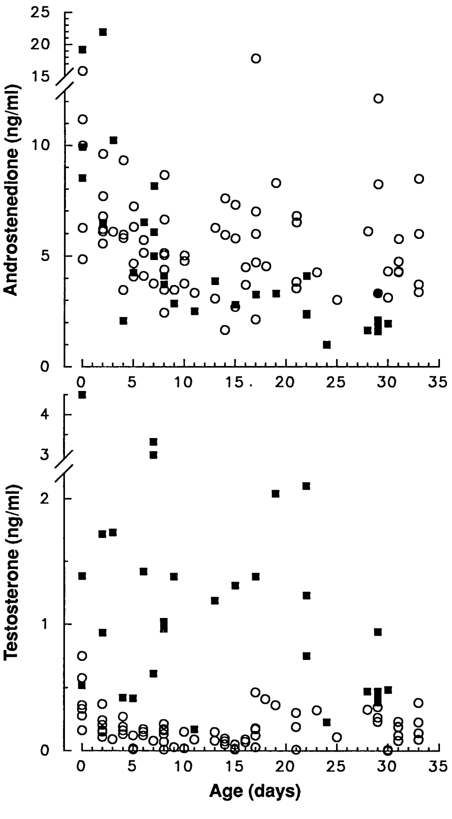

Fig. 3. Androgen levels in spotted hyenas during the first month of life. Males are represented by closed squares and females by open circles. For statistical analyses, multiple samples were averaged for each individual in four time periods: 0 to 3, 4 to 11, 12 to 19, and 20 to 33 days. Females displayed higher A levels on days 20 to 33 ($P < 0.01$), whereas males had higher levels of T ($P < 0.05$) at all age periods (Mann-Whitney *U* test).

Africa food supply may potentially limit hyena litter sizes; in the nearby Serengeti whole litters sometimes starve (*1*).

Competition for nutrition does not, however, explain the marked difference in survival between same- and mixed-sex litters. This phenomenon may be related to differential dispersal; that is, males disperse after puberty, whereas females remain in the maternal clan (*5*). Thus, mixed-sex twins are not competitors as adults, and the benefits gained from disposal of a sibling may not outweigh the costs of prolonged combat and the loss of inclusive fitness through siblicide. Competition within same-sex pairs may occur for different reasons. Rank affects reproductive success in both sexes (17). For females, elimination of a sister results in the removal of a close-ranking competitor, because females acquire the mother's rank in the social hierarchy (*5*). For males, confronting the difficult task of integration into a new clan, large size may be a particular advantage, and elimination of a brother does result in a more rapid weight gain during the first year of life (*15*).

Nonlethal intra-litter aggression occurs in neonatal domestic pigs and at a later age in several canids (*18*); this is the first report of habitual siblicide in a mammal. Siblicide appears to kill nearly 25% of spotted hyena offspring: at birth, 50% of litters are same sex, and half of those individuals succumb. Why does the mother apparently permit her offspring to reduce her own fitness? The fact that aggression was not fatal in captivity, where the mother had constant access to the neonates, suggests that the denning habit of spotted hyenas, whereby the infant pair is safe from predators, but out of reach of the female when not nursing, may permit aggression to proceed unchecked. The fatal outcome of fighting may thus be in part a result of ecological factors limiting potential parental influence on infant behavior, resolving parent-offspring conflict in favor of the offspring. Once aggression appeared, even if as a by-product of selection for some other complex of traits mediated by prenatal androgenization, immense advantage would accrue to the survivor, and there would be powerful selection for adaptations permitting "victory" in intra-litter combat, for example, precocial tooth eruption and general physical maturation (*19*).

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Linkage of a Cardiac Arrhythmia, the Long QT Syndrome, and the Harvey *ras*-1 Gene

MARK KEATING,* DONALD ATKINSON, CHRISTINE DUNN,
KATHERINE TIMOTHY, G. MICHAEL VINCENT, MARK LEPPERT

Genetic factors contribute to heart disease. In this study, linkage analyses have been performed in a family that is predisposed to sudden death from cardiac arrhythmias, the long QT syndrome (LQT). A DNA marker at the Harvey *ras*-1 locus (H-*ras*-1) was linked to LQT with a logarithm of the likelihood ratio for linkage (lod score) of 16.44 at $\theta = 0$, which confirms the genetic basis of this trait and localizes this gene to the short arm of chromosome 11. As no recombination was observed between LQT and H-*ras*-1, and there is a physiological rationale for its involvement in this disease, *ras* becomes a candidate for the disease locus.

CARDIOVASCULAR DISEASE IS A MAJOR cause of morbidity and mortality in the industrialized world. Over the last 10 years it has become increasingly apparent that inherited traits are involved in the pathogenesis of most cardiovascular disorders. Much attention has been and continues to be focused on the genes that regulate lipid metabolism and their role in atherogenesis. In this study, we have begun to investigate the genetic basis of a type of cardiovascular disease that is not directly linked to lipid abnormalities, that of cardiac arrhythmias.

Ventricular arrhythmias are a common cause of cardiac arrest and death (*1*). The pathogenesis of these arrhythmias is poorly understood, but predisposing factors include myocardial ischemia and infarction, metabolic abnormalities, and genetic factors. In this study we have examined a large

M. Keating, D. Atkinson, C. Dunn, Division of Cardiology, Department of Human Genetics and Eccles Program in Human Molecular Biology and Genetics, University of Utah Health Sciences Center, Salt Lake City, Utah 84132.
K. Timothy and G. M. Vincent, Department of Medicine, LDS Hospital, Salt Lake City, Utah 84143.
M. Leppert, Howard Hughes Medical Institute and Department of Human Genetics, University of Utah Health Sciences Center, Salt Lake City, Utah 84132.

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*To whom correspondence should be addressed.